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IMMUNOGENICITY OF BRUCELLA ABORTUS 19 STRAIN

IN TESTS ON GUINEA PIGS AND HUMAN BEINGS -- PRELIMINARY REPORT

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31 March 1947

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Investigations during recent years (Lisbonne, 1938; Tarasov, Striter, Mursalova, 1940-1941) have shown that inoculations with killed bacterial vaccines or their derivatives produced immunity against Brucellosis in a considerable number of animals. Experiments which we have conducted (1941-1945) with various types of killed vaccines in the immunization of guinea pigs have shown, however, that on the average only 40 percent of these animals acquired resistance to experimental *Brucella melitensis* infection. We furthermore established that such acquired immunity under those conditions was only relative. Thus, guinea pigs injected with a single virulent dose of *Brucella* showed 70-80 percent resistant and those given 5 to 10 virulent doses 40 percent resistant; whereas of those injected with 50 virulent doses, 95 percent became infected.

Furthermore, the immunity of inoculated guinea pigs is of short duration. The number of these animals retaining immunity decreased to one-third during the 3 months after inoculation. Consequently, inoculation with killed vaccines did not prove successful. However, we were able to produce re-

- 1 -

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sistance to five virulent doses of Brucella in 70 percent of these animals by immunizing them with avirulent living Brucella suis 22 vaccines. The immunogenicity in these animals was considerably greater, and 55 percent remained immune even after 50 virulent doses of Brucella were injected.

Having obtained these encouraging results with the avirulent strain of Brucella suis 22, we then took up the American strain, Brucella abortus 19, which is widely used in immunizing domestic animals.

We carried out the following tests in connection with this strain:

1. Determination of the virulence of this strain in guinea pigs.
2. Determination of the retention period of this strain in the tissues of the animals.
3. Determination of the immunogenicity of this strain in guinea pigs and also a tentative determination of the tolerance and immunogenicity of this strain in human beings.

A. Virulence of Brucella Abortus 19 Strain in Guinea Pigs

Guinea pigs weighing 350-400 grams were subcutaneously injected with a 48-hour culture of Brucella abortus 19 in doses containing from 1,000 to 2 billion bacterial count (according to the microscopic standard of TeGUKI). These inoculated animals were killed 30 days later and cultures were made from tissue specimens of the lymphatic glands, groin, parotid gland, maxilla and cervical foot, and also from the spleen, liver, blood, urine, and marrow. Results of these cultures are shown in Table 1.

Table 1

Doses	No of Guinea Pigs	Cultural Results	
		Positive	Negative
1,000	6	0	6
100,000	4	4	0
1,000,000	2	2	0
100,000,000	2	2	0
1,000,000*	6	6	0
2,000,000,000	5	5	0

* [sic; probably should be 1,000,000,000]

Brucella abortus 19 strain shows less virulence in guinea pigs. However, its "residual" virulence remains sufficiently apparent insofar as a dose totalling 100,000 bacterial count produced a generalized infection in these animals. It is also interesting to note that we failed to observe either clinical or pathological degenerations in those animals injected with Brucella abortus 19 strain.

B. Retention Period of Brucella Abortus 19 Strain in the Tissues of Guinea Pigs

Guinea pigs were subcutaneously injected with Brucella abortus 19 strain culture containing a dose of one billion bacterial count. Pathologic anatomy and bacteriological examination of these animals were made at various intervals after the injection. Results of these examinations are shown in Table 2.

- 2 -

RESTRICTED

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Table 2

Time of Pathologic Anatomy	In days					In months			
	3-5	5-10	15-20	30-35	2	3	5	6	
No of guinea pigs examined	5/5	6/6	19/19	7/6	50/24	21/5	19/2	29/1	
Percent of cultures obtained	100.0	100.0	100.0	85.0	48.0	23.8	10.5	3.5	

NOTE: Numerator shows number of guinea pigs examined; denominator shows number of cultures obtained.

These experiments indicated in this manner that a certain number of these animals showed signs of recovery from *Brucella abortus* 19 infection in about 30 days and in 6 months they became practically immune to *Brucellosis*. Cultures were made generally from lymph fluid in animals which were killed 2-3 months after the injection, but after 6 months it was possible to obtain cultures only from the urine specimens of one animal out of 29. Cultural growths made from sections of the vital organs of the animals were insignificant and were only in isolated colonies on agar. Cultures often showed propagation in broth only, i.e., due to increased nutritive value.

C. Immunogenicity of *Brucella Abortus* 19 Vaccine in Guinea Pigs

Guinea pigs were subcutaneously injected with living *Brucella abortus* 19 vaccine with a dose containing a one billion bacterial count. Due to this injection, the animals showed immunobiological reactions (Wright reaction, opsonic, phagocytic, and allergic tests). These immunized animals were later divided into four groups and were then injected with various dosages of virulent *Brucella melitensis* (437) at various intervals after the original vaccination.

After 30 days, the first group of immunized animals was injected with one dose of *Brucella* and the second with five doses. Simultaneously, control animals were injected with identical doses of *Brucella*. The third group was injected with one dose 2 months after immunization. Finally, 4 months after immunization, a fourth group was injected with killed vaccine having a 2 billion bacterial count. Thirty days after the second injection these animals were injected with a highly virulent strain isolated from the udder of an aborted sheep.

The following pathologic anatomy and bacteriological analyses of the animals in all the groups were made 30-35 days after the injection of the virulent strain. Cultures from each organ were differentiated according to their formation of H₂S and/or producing aniline in the culture media. The results are shown in Table 3.

- 3 -

RESTRICTED

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Table 3

No of Inoculated Animals	Amt of Virulent Dose	No of Days after Inoculation Before Signs of Infection Appeared	No of Animals Infected	No of Animals Injected With Br. Abortus 19 (showing immunity to infection)	No of Non-infected Animals (showing acquired immunity)
14	1	30	0	9	5
18	5	30	0	9	9
21	1	60	3	4	14
Revaccinated animals					
29	1	150	1	1	27
Control					
42	1-5	-	42	-	-

Table 3 shows that animals inoculated with *Brucella abortus* 19 vaccine were 100 percent immune to *Brucella melitensis* after a month and 50-64 percent were due to acquired immunity. Those animals which showed no trace of *Brucella abortus* 19 vaccine (55-50 percent) soon after inoculation nevertheless showed no sign of infection and apparently were in a period of acquired immunity. Two months after inoculation 84 percent of the animals already showed immunity against *Brucella melitensis*, and the majority of these (about 64 percent) showed no infection. However, 14 percent of the animals have apparently lost their acquired immunity by this time. Finally, revaccination tests proved that it was simple to "reactivate" acquired immunity in 4 months after the first inoculation by revaccination with killed vaccine. Ninety-seven percent of the animals acquired resistance to virulent *Brucella* injections through this method of revaccination.

The results of the immunological reactions in animals inoculated with *Brucella abortus* 19 vaccine are shown in Table 4.

Table 4

Time After Inoculation	Avg Titer for Wright Reaction Test	Avg Index of Opsonic Phagocytic Reactions	Positive Reaction Biurue (in %)
3 days	1:320	30	44
60 days	1:290	65	40
4 months	1:40	27	67

Table 4 shows a considerable decrease in the agglutination titer and also the index of opsonic phagocytic reaction in the animals after 4 months. On the other hand, the Biurue reaction shows an increase tendency which reached its maximum (67 percent) after 4 months in our experiments.

D. Vaccination of Human Beings with *Brucella Abortus* 19 Strain

At the end of last year (1946), we inoculated a few volunteers with living *Brucella abortus* 19 vaccine after testing this vaccine on guinea pigs. We chose volunteers who were negative to *Brucella* (Wright, Edelson, opsonic phagocytis, and Biurue reaction tests) and free of other undesirable factors to hinder this inoculation test. These men ranged from 23 to 43 years. The vaccine was prepared from a 48-hour culture of

- 4 -

RESTRICTED

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Brucella abortus 19 in physiologic salt solution. A 1-cc dose was injected subcutaneously into the forearms. Following this injection, local and general reactions, as well as the immunobiological reactions were studied, in addition to the careful observation of the general condition of the volunteers. As a result of this experiment, we were able to determine the tentative immunogenic doses of *Brucella abortus* 19 vaccine for human beings. As an example, we could mention the following data obtained from two of the volunteers (K and V) who were inoculated with various doses of *Brucella abortus* 19 vaccine.

Subject K was injected with a dose equivalent to 1,000 virulent doses for a guinea pig while subject V was injected with twice this amount. Both showed insignificant local reactions and there was slight inflammation in the injected sites after 24 hours with very little pain lasting for 2-3 days. Following the injection, K had a temperature of 37.2 and V had 37.1 in 36 hours. On the injection day and the following days these men performed their full quota of work as usual.

Study of the immunobiological reactions in inoculated cases determined the following results:

K showed negative Wright reaction 15 days after inoculation, but showed positive Huddelson reaction in 1:50 titration. V likewise showed negative Wright reaction, but the Huddelson reaction was well defined in 1:200. Blood cultures were negative. K had a positive Wright reaction in 1:50 titration after 30 days, and positive Huddelson reaction in 1:200 titration, and a strong positive Biurne reaction. For the same period, V showed positive Wright and Huddelson reactions in 1:200 titration after his double dose of vaccine, and positive Biurne reaction also. Further study of immunobiological reactions of these inoculated volunteers showed that they responded to serological and allergic tests two months after inoculation. Finally, in the course of observation of the general health of these inoculated volunteers, we found that their temperatures were below fever level within 19-21 days after inoculation with a maximum of 37.3 and remained at this level for 23-25 days. Both subjects were feeling quite well.

The preliminary experiments on human beings have shown in this manner that the strain of *Brucella abortus* 19 (American) was quite tolerant as far as human beings were concerned, and indicated apparently good immunogenicity.

Thus, the first tentative experiment warrants a continuation of this work in order to determine conclusively the effectiveness of inoculation in human beings with *Brucella abortus* 19 vaccine against Brucellosis.

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- 5 -

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